REMARKS

1. Response to the Restriction Requirement

The Examiner has required in the Office Action dated October 22, 1999, that pursuant to 35 U.S.C. §121 and 37 C.F.R. §1.142, the subject application be restricted to one of the alleged two "inventions" of Group I (Claims 1-29) and Group II (Claims 30-43). Responding thereto, Applicants provisionally elected, with traverse, the Group I invention presently defined by Claims 1-29. Applicants respectfully request reconsideration and withdrawal of the restriction requirement.

The Examiner states that the proteins of Group II are related to the method of Group I because expression of the proteins is required for practice of the method. However, the Examiner maintains that the Claims of Group I and Group II are distinct each from the other because the proteins can be produced by other means, such as naturally or by chemical synthesis and because the proteins can be used for purposes other than therapy, such as antibody production or functional investigation.

The Examiner is respectfully requested to reconsider the restriction requirement. It is not readily understood that examining the alleged two Group inventions would cause a serious burden on the PTO. On the other hand, the alleged two Group inventions resulting from the restriction requirement would cause Applicants additional expenses and time to prosecute separate patent applications. The burden placed upon Applicants for filing separate cases directed to each of the groups is respectfully submitted not to be outweighed by the burden placed upon the Patent Office by a non-coextensive examination of these groups in one application. Consequently, in the interest of efficiency, it is respectfully submitted that the restriction requirement is untenable and ought to be withdrawn.

The Examiner is respectfully requested to further reconsider the restriction requirement under 35 U.S.C. §121 to elect a single invention. The requirements of §121 are that the inventions be independent and distinct. Both requirements are necessary to maintain a restriction requirement. Applicants respectfully maintain that the Examiner has not focused upon the two requirements of being independent and distinct. It is noted that

M.P.E.P. §802.01 provides a definition of independent as follows:

> The term "independent" [i.e., not dependent] means that there is no disclosed relationship between the two or more subjects disclosed, that is, they are unconnected in design, operation or effect, for example, [1] species under a genus which species are not usable together as disclosed or [2] process and apparatus incapable of being used in practicing the process.

Applicants agree with the Examiner that the claimed method for producing and delivering of protein in vivo (Claims 1-29, Group I) and a protein produced and delivered in vivo by a process (Claims 30-43, Group II) are distinct from each other. However, the group inventions are not independent of each other. The proteins of Group II are related to the method of Group I because expression of the proteins is required for practice of the method. More specifically, the claimed proteins defined in Claims 30-43, Group II are produced by the method defined in Claims 1-29, Group I. Therefore, Groups I and II are related and not independent from each other.

For the above reasons, reconsideration of the restriction requirements for the alleged two Group inventions set for the in the outstanding Office Action is respectfully requested.

2. Response to Rejection of Claims 10 and 24 based on 35 U.S.C. §112

Claims 10 and 24 stand rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains to make and/or use the invention. The Examiner asserts that neither the prior art nor the specification discloses a method for selectively lysing only red blood cells which comprise a protein of interest. The Examiner further suggests that the method is asserted to be therapeutic, it is assumed that general lysis of all red blood cells in an organism is not acceptable. This rejection is respectfully traversed.

Claim 10 and 24 are dependent claims depending on claim 1 and 16, respectively. Claim 1 is a method for producing a non-native protein in the progenitor

cells of red blood cells, and using red blood cell rupture to deliver produced protein into blood stream in vivo. Claim 16 a method using a hemoglobin promoter to produce a non-hemoglobin protein in the progenitor cells of red blood cells, and using red blood cell rupture to deliver produced protein into blood stream in vivo. Claims 10 and 24 more particularly define the claimed invention to a method of delivering proteins in which red blood cells containing the proteins are induced to rupture in vivo by genetic mutation.

Applicant disclosed in the specification (page 12, line 2) that the life cycle time of red blood cells can be modified by genetic mutation. Thus, as appreciated by one skilled in the art, the shortening of the red blood cells life cycle by genetic mutation is an example of induced rupture. Applicant made such an example because genetic mutation can cause shortening of red blood cell life time. For instance, hereditary spherocytosis and elliptocytosis are human disease resulting from genetic mutation. In both cases, abnormally shaped red blood cells have shorter life time. It is also known that mutations of red blood cell enzymes lead to haemolytic anemia. Copies of two references regarding genetic mutations are enclosed in the Response. Furthermore, it is understood by one skilled in the art that mutations of cytoskeletal proteins and red blood cell enzymes can be accomplished by genetic engineerings.

On the other hand, since the promoter, particularly a hemoglobin promoter, is only active in progenitor cells of red blood cells, the shortening cell life cycle, or induced rupture are limited only to red blood cells which contain the expressed protein. Therefore, the induced rupture does not occur to any other cells, including red blood cells or white blood cells, which are not descendent cells of the progenitor cells containing the mutated gene. More specifically, the life cycle of normal red blood cells, which are descendent cells of normal progenitor cells without the inserted mutated gene, will not be disturbed.

Applicant understands the Examiner's concern on general lysis of all red blood cells in an organism being not acceptable. In this aspect, the present claimed invention has specific advantages not shared by the prior art. Applicant respectfully recites the discussion in the specification as filed (page 12, line 7-25):

Fifth, with the method of the present invention, the amount of protein production and delivery can be controlled by the amount of host cells collected and treated. When the stem cells are collected from bone marrow, the treated bone marrow can be implanted at the original collection position, or enclosed in a sag, then implanted back into a patient's bone marrow. If a large quantity of protein is needed, such as human serum albumin, the amount of protein production can be controlled by the numbers of transplant sites. If a patient inherits a genetic defect and needs a continuing supply of the normal gene product throughout life, a permanent implantation could be selected. If only short term activity of a gene is needed, such as to activate the immune system against cancer cells or an infectious agent, the sag can be taken out from the body when the therapy is complete, or no longer desired.

Sixth, with the method of the present invention, the protein is only present in red blood cells. Once treated stem cells are taken out from the body, their red blood cell production will stop instantly. The remaining red blood cells in the peripheral blood, which carry the protein, will rupture at the end of their life cycle. By then, the supply of the protein will stop completely. Therefore, the method of the present invention provides a simple safety control mechanism for the therapeutic process.

..... since red blood cells do not produce protein in the peripheral blood, the protein production is confined in the parent stem cells, which can be controlled by the amount of transplant.

Therefore, general lysis of all red blood cells, as speculated by the Examiner, will not occur by employing the method of the present invention.

Accordingly, Applicant respectfully requests withdrawal of the rejection of Claims 10 and 24 based upon 35 U.S.C. §112, first paragraph.

3. Response to Rejection of Claims 1-29 based upon 35 U.S.C. §112

Claims 1-29 stand rejected under 35 U.S.C. §112, first paragraph. The Examiner asserts that the specification, while being enabling for delivery of a protein to the blood in vivo, and to a cell expressing receptors for the protein, does not reasonably provide enablement for delivery of a protein to a cell which lacks receptors for the protein, nor does it provide enablement for therapy based on the delivery of any protein. The Examiner contends that the specification does not enable a person skilled in the art to use the invention commensurate in scope with these claims. This rejection is respectfully traversed.

Claim 1 of the present invention is a method for producing and delivering protein in vivo. More specifically, the method is to produce a non-native protein in the progenitor cells of red blood cells, and using red blood cell rupture to deliver produced protein into blood stream in vivo.

Claim 16 of the present invention is a method using a hemoglobin promoter to produce a non-hemoglobin protein in the progenitor cells of red blood cells, and using red blood cell rupture to deliver produced protein into blood stream in vivo.

Applicant respectfully points out that the present claimed invention is to a method of producing a protein only in the progenitor cells of red blood cells, and delivering produced protein into blood stream by rupture of the red blood cells. From the blood stream, the protein can be delivered to the functional site, which can be directly in the circulating blood, as in the case of human serum albumin and insulin, or to specific organs and tissues. However, Applicant's claimed invention is not a mechanism of protein intake by a specific cell. Furthermore, although the present invention can be used for disease treatment as apparent to one skilled in the art, Applicant claimed invention as defined by the claims is not, nor intended to be, a specific gene therapy protocol.

Applicant is only responsible for enablement of the disclosed method within the scope defined by the claims, not beyond the scope of the claims. As Examiner stated in the above citation (page 4, line 6 of the Office Action), the present invention is "enabling for delivery of a protein to the blood in vivo". That is precisely the claimed invention.

Therefore, Applicant believes that the specification, as filed, satisfies the enablement requirement.

Accordingly, Applicant respectfully requests withdrawal of the rejection of Claims 1-29 based upon 35 U.S.C. §112, first paragraph.

4. Response to Rejection of Claims 1, 3, 5-9, 11, and 13 based upon 35 U.S.C. §102(b)

Claims 1, 3, 5-9, 11, and 13 stand rejected under 35 U.S.C. §102(b) as being anticipated by Schlegel (US Patent 5,576,206). This rejection is respectfully traversed.

Schlegel teaches a process of immortalizing cells with isolated HPV-16, 18, 31, 33 or 35 E6 and E7 genes or E7 gene alone to produce non-tumorigenic immortalized cell lines which retain the differentiated phenotypic characteristics of the parent cells.

Schlegel further teaches a method of gene therapy in which cells from patient are removed from the patient and immortalized to produce non-tumorigenic immortalized cells retaining the differentiated phenotypic characteristics of the original parent cells, and then reintroducing the immortalized cells into the patient as a therapeutic treatment (column 3, line 22-28). Immortalized host cells which retain the phenotypic characteristics of the parent cells are not immunogenic the host immune system and therefore useful for gene therapy (column 4, line 6-9). Epithelial cells are particular preferred host cells for immortalization using this method (column 6, line 56-60).

Furthermore, Schlegel teaches that alternative to infusion, immortalized cells may be directly injected with the appropriate target organ. For example, immortalized prostate cells may be directly injected into the prostate to establish foci of genetically engineered cells (column 9, line 60-64).

Applicant's claimed invention is a method for producing and delivering protein in vivo, which comprises inserting a promoter and a gene encoding a non-native protein to red blood cells in a vector, wherein said promoter is active only in the progenitor cells of red blood cells; collecting an amount of progenitor cells of red blood cells from a mammal; treating said progenitor cells of red blood cells in vitro with said vector; introducing the treated progenitor cells of red blood cells back to said mammal, wherein

the treated progenitor cells of red blood cells produce red blood cells and said protein in vivo in said mammal, and wherein said protein is contained only in said red blood cells, and thereafter said protein is released into blood stream of said mammal through rupture of said red blood cells.

Schlegel is a deficient reference in two aspects. First, Schlegel fails to teach Applicant's claimed producing a non-native protein only in the progenitor cells of red blood cells in vivo. Second, Schlegel fails to teach Applicant's claimed utilizing red blood cells as a vehicle for delivering produced protein into blood stream in vivo through the rupture of the red blood cells.

As to the second aspect, the Examiner asserts although Schlegel does not teach that red blood cells should lyse and release the protein into the blood, lysis of red blood cells is an inherent property, therefore, Schlegel anticipates Applicant's claimed invention. Applicant respectfully disagrees.

It is known to one skilled in the pertinent art that in natural protein production and delivery process, after completion of protein synthesis in a cell, the proteins will either be retained in the cell or will be transported from their manufacture sites to destined functional sites. There are two pathways for proteins to export from the cells. The first pathway is constitutive secretion. The second pathway is exocytosis. Both process are natural processes of protein exportation.

Thus, Schlegel teaches that the immortalized cells are either actual functional sites of the gene expression product, or they export the product through natural pathways, which is contrary to Applicant's claimed invention. To illustrate, Applicant specifically points out Schlegel's disclosure in column 9, line 29-41 as follows:

> Although any donor gene may be used which is capable of expressing a product in and secreting it from endothelial cells, mention may be made of several non-limitative examples.
>
> For example, cells in tumor metastasis deposits may secrete cytokines ....., or alternatively secrete agents which could interfere with further angiogenesis, such as growth factor receptor-blocking peptides

Therefore, these immortalized cells are not utilized as a carrier to deliver proteins into the blood stream to other functional sites through the rupture mechanism claimed by Applicant. It is apparent from above discussion that Applicant's claimed method for delivering protein in vivo by rupture of the red blood cell bypasses the two natural protein exportation pathways. Consequently, the mechanism of protein delivery by the method of the present invention is fundamentally different from natural cell secretion or exocytosis taught by the prior art. Moreover, without recognizing that rupture of red blood cells can be utilized for protein delivery, Schlegel fails to anticipate any mechanism for controlling production of a non-native protein only in the progenitor cells of red blood cells. As a result, Schlegel fails to anticipate or imply Applicant's claimed invention.

Accordingly, Applicant respectfully requests withdrawal of the rejection based upon 35 USC §102(b).

5. Response to Rejection of Claims 1, 2, 4, 7, 11-23, and 25-29 based upon 35 U.S.C. §103 (a)

Claims 1, 2, 4, 7, 11-23, and 25-29 stand rejected under 35 U.S.C. §103 (a) as being unpatentable over Schlegel (US Patent 5,576,206) in view of Carrano (US Patent 5,739,118), Wickham et al (US Patent 5,846,782), Rixon et al (Mol. Cell. Biol. 8(2): 713-721, 1988), Zhang et al (Shengwu Huaxue Zazhi 11(3): 343-347, 1995), and Chatterjee et al (US Patent 5,935,821). This rejection is respectfully traversed.

Claim 1 and 16 are independent claims with different claim limitations. Claim 1 of the present invention is directed to a method for producing a non-native protein in the progenitor cells of red blood cells, and using red blood cell rupture to deliver produced protein into blood stream in vivo. Claim 16 of the present invention is directed to a method using a hemoglobin promoter to produce a non-hemoglobin protein in the progenitor cells of red blood cells, and using red blood cell rupture to deliver produced protein into blood stream in vivo. As discussed previously, the claimed method of the present invention for delivering protein in vivo bypasses the two natural protein

exportation pathways. Instead, the claimed method utilizes red blood cell rupture, as the mechanism to deliver desired proteins into the blood stream.

Schlegel teaches a process of immortalizing cells with isolated HPV-16, 18, 31, 33 or 35 E6 and E7 genes or E7 gene alone to produce non-tumorigenic immortalized cell lines which retain the differentiated phenotypic characteristics of the parent cells.

Schlegel fails to teach producing a non-native protein only in the progenitor cells of red blood cells. Moreover, Schlegel fails to teach Applicant's claimed utilizing red blood cells as a vehicle for delivering produced protein into blood stream in vivo through the rupture of the red blood cell. In addition, Schlegel fails to teach using a hemoglobin promoter to control gene expression and production of non-hemoglobin proteins in the progenitor cells of red blood cells. Schlegel also fails to teach utilizing red blood cells as a vehicle for delivering the produced non-hemoglobin proteins into blood stream in vivo through the rupture of the red blood cell. The deficiencies of Schlegel are not overcome by the Examiner's picking and choosing of selected teachings from the additional references of Carrano et al., Wickham, Rixon, Zhang and Chatterjee.

Carrano et al. teach methods of introducing genetic material into the cells of an individual. The methods comprises the steps of contacting cells of an individual with a genetic vaccine facilitator agent and administering to the cells, a nucleic acid molecule that comprises a nucleotide sequence that either encodes a desired peptide or protein, or serves as a template for functional nucleic acid molecules. The nucleic acid molecule is administered free from retroviral particles (column 1, line 52-67).

Carrano et al. further teach that the gene construct may be administered directly into the individual to be immunized or ex vivo into removed cells of the individual which are reimplanted after administration. By either route, the genetic material is introduced into cells which are present in the body of the individual (Column 12, lines 63-65).

It would be inconsistent to combine Schlegel with Carrano et al. as proposed by the Examiner for the purpose of delivering expression vectors to the cells ex vivo, wherein the expression vector utilizes a hemoglobin promoter and an enhancer. More specifically, Schlegel utilizes a viral vector whereas Carrano et al. incorporates genetic constructs without retroviral particle-mediated insertion. Therefore, one skilled in the art

would not be motivated to combine the two references in the manner suggested by the Examiner.

Moreover, even if one skilled in the art were to combine the references, it would not make Applicant's claimed invention obvious. More specifically, Carrano et al. only teach methods, compositions and kits for introducing genetic material into cells, and rely on the natural pathways of the reimplanted cells for exporting the gene expression product in vivo. Carrano et al. fail to teach producing a non-native protein only in the progenitor cells of red blood cells, and delivering the gene expression product into blood stream in vivo through rupture of red blood cells. Furthermore, Carrano et al. fail to teach using hemoglobin promoter to produce non-hemoglobin proteins in the progenitor cells of red blood cells, and using rupture of the red blood cells as the mechanism for delivering the produced non-hemoglobin proteins into blood stream in vivo.

Wickham teaches a chimeric adenovirus fiber protein, which differs from native protein by the introduction of a nonnative amino acid sequence in a constrained manner. The introduction gives the resultant chimeric adenovirus fiber protein an ability to directly entry into cells of a vector more efficiently. Wickham also teaches vectors that comprise the chimeric adenovirus fiber protein, and the methods of constructing and using such vectors (column 3, line 46-61). Wickham's teaching provides improved vectors and methods for cell targeting. Wickham's chimeric adenovirus fiber protein and method of use only addresses efficiency of protein production by improving vectors and methods for cell targeting, through increasing the affinity of a peptide for a cell surface binding site (column 73, line 1).

Wickham et al. fail to teach producing a non-native protein only in the progenitor cells of red blood cells, and delivering the gene expression product into blood stream in vivo through rupture of red blood cells. Furthermore, Wickman et al. fail to teach using hemoglobin promoter to produce non-hemoglobin proteins in the progenitor cells of red blood cells, and using rupture of the red blood cells as the mechanism for delivering the produced non-hemoglobin proteins into blood stream in vivo. Consequently, using lentiviral vector for gene expression is not relevant to Applicant's claimed invention.

Rixon teaches a mutated hemoglobin promoter, Greek-type gamma HPFH promoter, having increased activity relative to the wild type. Rixon's teaching addresses

efficiency of gene expression, however, it does not address the issue of delivery of a gene expression product to its functional site after its production.

Rixon et al. fail to teach producing a non-native protein only in the progenitor cells of red blood cells, and delivering the gene expression product into blood stream in vivo through rupture of red blood cells. Furthermore, Rixon et al. fail to teach using hemoglobin promoter to produce non-hemoglobin proteins in the progenitor cells of red blood cells, and using rupture of the red blood cells as the mechanism for delivering the produced non-hemoglobin proteins into blood stream in vivo.

Zhang et al. also teach mutated hemoglobin promoter taught by Rixon having increased activity relative to the wild type. Zhang et al. suggest to transfer the A. gamma. 117 globin gene into human hematopoitic stem cells for the purpose of ameliorating certain types of anemia. Again, Zhang et al 's teaching only addresses efficiency of gene expression. More specifically, in the suggested appropriate applications the descendent cells of hematopoitic stem cells are actual functional site of the gene expression product. The hemoglobin produced in such method only functions in red blood cells, and there is no need to deliver the protein outside of red blood cells.

Zhang et al. fail to teach producing a non-native protein only in the progenitor cells of red blood cells, and delivering the gene expression product into blood stream in vivo through rupture of red blood cells. Furthermore, Zhang et al. fail to teach using hemoglobin promoter to produce non-hemoglobin proteins in the progenitor cells of red blood cells, and using rupture of the red blood cells as the mechanism for delivering the produced non-hemoglobin proteins into blood stream in vivo.

Applicant's claimed invention utilizes a hemoglobin promoter to produce a non-hemoglobin protein in the progenitor cells of red blood cells in vivo. Rixon and Zhang teach using a mutated hemoglobin promoter to improve expression efficiency of hemoglobin protein. Therefore, the two prior art teach away from the Applicant's claimed invention. More specifically, Rixon and Zhang fail to teach delivering produced non-hemoglobin protein into blood stream by red blood cells through their rupture.

Chatterjee et al. teach monoclonal antibody 1A7, and polynucleotide and polypeptide derivatives based on 1A7. When administered to an individual, the 1A7 antibody overcomes immune tolerance and induces an immune response against

ganglioside GD2. In addition, Chatterjee et al. teach although expression of the nucleic acid is involved when the polynucleotides encoding the antibody 1A7 are used for immunization, exportation of the expression product relies on cells' natural secretion.

Chatterjee et al. fail to teach producing a non-native protein only in the progenitor cells of red blood cells, and delivering the gene expression product into blood stream in vivo through rupture of red blood cells. Furthermore, Chatterjee et al. fail to teach using hemoglobin promoter to produce non-hemoglobin proteins in the progenitor cells of red blood cells, and using rupture of the red blood cells as the mechanism for delivering the produced non-hemoglobin proteins into blood stream in vivo.

In conclusion, viewing all the teachings of the prior art presented, one of ordinary skill in the art would not have expected to be able to modify or combine the Schelgel, Wickham, Carrano, Rixon, Zhang, and Chatterjee references to obtain Applicant's claimed invention.

On the other hand, it has been a long felt need for solutions to the problem of production and delivery of protein in vivo. New strategies and methods to overcome difficulties in achieving the goals of producing and delivering proteins in vivo are strongly in demand. The fact that lack of teaching in the art, either patents or scientific publications, on using Applicant's approach for protein production and delivery in vivo strongly indicates unobviousness of Applicant's claimed invention. Therefore, Applicant firmly believes that Applicant's claimed invention is unobvious in view of the prior art. Accordingly, Applicant respectfully requests withdrawal of the rejection based upon 35 U.S.C. §103(a).

It is respectfully submitted that Claims 1-29 are now in condition for allowance and such action is respectfully submitted. Applicant's Agent respectfully requests direct telephone communication from the Examiner with a view toward any further action deemed necessary to place the application in final condition for allowance.

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